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Cloning and molecular analysis of poly(3-hydroxyalkanoate) biosynthesis genes in Pseudomonas aureofaciens.

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Pseudomonas aureofaciens grown on octanoate or gluconate synthesized medium-chain-length polyhydroxyalkanoates (mcl-PHAs). To clone the PHA synthase gene(s) (phaC), the genomic library of P. aureofaciens was constructed using a cosmid vector. The recombinant cosmids that clone phaC were detected by the complementation with a PHA-negative mutant, P. putida GPp104. The resulting recombinant cosmid, named pVK6, contained a 13-kbp DNA insert. Genetic analysis of the pha locus in pVK6 revealed the presence of six ORFs, genes encoding two PHA synthases, 1 and 2 (phaC1 and phaC2), PHA depolymerase (phaZ), two PHA granule-associated proteins (phaF and phaI), and an unknown protein (phaD). The heterologous expression of pha genes from P. aureofaciens was confirmed. P. putida GPp104 regained the ability to accumulate PHA on introduction of pVK6. Wild-type strains P. oleovorans and P. fluorescens, which were unable to accumulate PHA when grown on gluconate, acquired the ability to accumulate PHA from gluconate when they possessed pVK6.

PMID: 11815858 [PubMed - indexed for MEDLINE]

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